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The Relation between Dosage and Death-Time

BY

A. T. GLENNY

Wellcome Physiological Research Laboratories, Beckenham, Kent

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A. T. Glenny.

Wellcome Physiological Research Laboratories, Beckenham, Kent.

Although it is of little practical value to the immunologist yet the relation between the amount of toxin injected into an animal and the time of death resulting is of considerable theoretical interest. The various connecting formulæ that have been suggested in the past were reviewed (Glenny, 1914) in a criticism of the formula suggested by Dreyer and Walker (1914)

$$\frac{1}{D_0 - a} - \frac{1}{D_1 - a} = k(T_0 - T_1)$$

when D_0 and D_1 are "surface doses" corresponding to the times T_0 and T_1 in which the death of the animal takes place, and a and k are constants for the particular toxin and species of animal used. It was then stated that "experimental evidence published in support of their formula is by no means conclusive and could be applied equally well in support of other formulæ."

A consideration of other formulæ suggested by different observers showed them all to be modifications of the general formula $(D - a)^m(T - b) = c$ when D and T are corresponding doses and death-times and a and b are the theoretical minimal lethal dose and minimal lethal time respectively. It was pointed out that for biological reasons $(T - b)$ is more likely to be a function of $(D - a)$ or of (D/a) than of D alone, and that either of the two formulæ $(D - a)^m(T - b) = C$ or $(\log D - \log a)(T - b) = C$ was the simplest form of such relationship which was likely to be adequate in practice.

Considerable experimental data were prepared and collected with the object of testing these formulæ by data, obtained experimentally, of the lethal times for a wide range of doses of diphtheria toxin and of cobra venom injected into guinea-pigs. In the former paper it was pointed out that over a short range of doses many formulæ fitted equally well and that in order to decide definitely in favour of any one formula it was necessary to examine the results from a wide range of doses.

If the minimal lethal dose (a) and the minimal lethal time (b) can be obtained experimentally it is a simple matter to test any of the alternative formulæ. If the lethal times (T) for a number of different doses (D) are known then it is necessary to determine whether $(T - b)$ multiplied by a certain function of $(D - a)$ (according to the particular formula being tested) gives a fairly constant value. It is however possible to obtain approximate indication of the values for (a) and (b) by direct experiment.

Diphtheria toxin.

Dose and death-time.

In the first experiments recorded below guinea-pigs weighing 250 grms. were used and all injections were made within a period of a few weeks during summer time. For accurate comparison between

size of dose and time of death, the period during which comparison is made must be limited because it is already known that there is a seasonal variation in susceptibility of guinea-pigs to diphtheria toxin (Glenny and Sudmersen, 1910). Table I. shows the results of injecting

TABLE I.

Observed lethal times in hours, of 73 *guinea-pigs of* 250 *grams weight injected with different doses of diphtheria toxin* J 2617. *The average lethal times are compared with times calculated according to the formula* $\log\left(\frac{D}{0{\cdot}002}\right)(T-10)=12$.

Dose.	0·001.	0·0015.	0·002.	0·003.	0·004.	0·005.	0·01.	0·05.	0·5.	5·0
Observed lethal times	lived	lived	lived	123·0	62·0	48·0	32·0	25·5	17·5	13
	,,	,,	,,	103·5	35 0	47·0	31·0	25·1	17·4	12
	,,	,,	180·0	102·6	...	44·3	28·0	24·0	17·2	10
	,,	96·0	135·0	55·0	...	44·0	24·7	23·5	16·5	10
	...	...	96·0	49·5	...	38·0	24·7	23·0	16·2	9
	...	...	84·0	47·0	...	36·8	22·7	...	16·2	9
	...	...	70·7	...	...	34·0	...	...	16·7	..
	...	...	67·0	...	...	31·0	...	22·9	15·2	..
	...	...	...	...	...	30·0	...	22·2	14·7	..
	...	...	...	...	...	29·0	...	21·0	15·7	..
	...	...	...	...	...	24·0	...	21·0	7·5	.
	...	...	...	...	...	23·0	...	21·0	...	.
	...	...	...	...	...	...	...	20·7	...	.
	...	...	...	...	...	...	...	13·0	...	.
	...	...	...	...	...	...	...	12·0	...	.
Average lethal times	lived	lived	105·4	80·1	48·5	35·7	27·2	21·1	15·3	1(
Calculated lethal times	,,	,,	lived	78·2	49·9	40·1	27·2	18·6	14·4	1

a toxin of average strength into 73 guinea-pigs of 250 grams weigh A consideration of the results shows that the M.L.D. of the toxin i about 0·002 c.c.: of 8 guinea-pigs injected with this dose 4 died in les than 5 days, 2 died later and 2 survived. Guinea-pigs injected wit 2500 fatal doses died in from 9 to 13 hours; the minimal lethal tim may therefore be taken to be about 10 hours or less.

No formula of the nature of

$$\frac{1}{D_0 - a} - \frac{1}{D_1 - a} = k(T_0 - T_1),$$

with a value of (a) approximating to 0·002 c.c. can satisfy the observe average lethal times of 21·1, 15·3 and 10·8 hours respectively for dos of 0·05 c.c., 0·5 c.c. and 5·0 c.c. The formula

$$(\log D - \log a)(T - b) = C$$

was therefore tried and, accepting round values for the constant, v found that

$$(\log D - \log 0{\cdot}002)(T - 10) = 12$$

fitted observed figures sufficiently closely. A more exact choice constants would no doubt produce a formula by which certain observ

times would agree more closely with the calculated times, but it must be remembered that so many other factors are involved besides the pathological effect of the pure poison that no simple formula could allow for all variables.

Table II. gives corresponding results with 32 guinea-pigs injected with a strong toxin concentrated by the method of Glenny and Walpole

TABLE II.

Observed lethal times in hours, of 32 *guinea-pigs of* 250 *grams weight injected subcutaneously with different doses of concentrated diphtheria toxin* JC 2538. *The average lethal times are compared with times calculated according to the formula* $\log\left(\frac{D}{0{\cdot}00008}\right)(T-10)=12$.

Dose.	0·00005.	0·000075.	0·0001.	0·00025.	0·0005.	0·005.	0·05.	0·5.	5·0.
Observed lethal times	lived	lived	156·0	41·4	25·5	24·0	16·7	17·0	12·5
	,,	,,	120·0	33·0	23·7	23·0	15·5	16·0	10·5
	,,	134·0	105·0	...	23·0	19·5	14·0	11·0	...
	,,	90·0	86·0	...	22·2	15·0	13·0	10·0	...
Average lethal times	,,	lived	116·8	37·2	23·6	20·4	14·8	13·5	11·5
Calculated lethal times	,,	,,	133·8	34·2	25·1	16·7	14·3	13·2	12·5

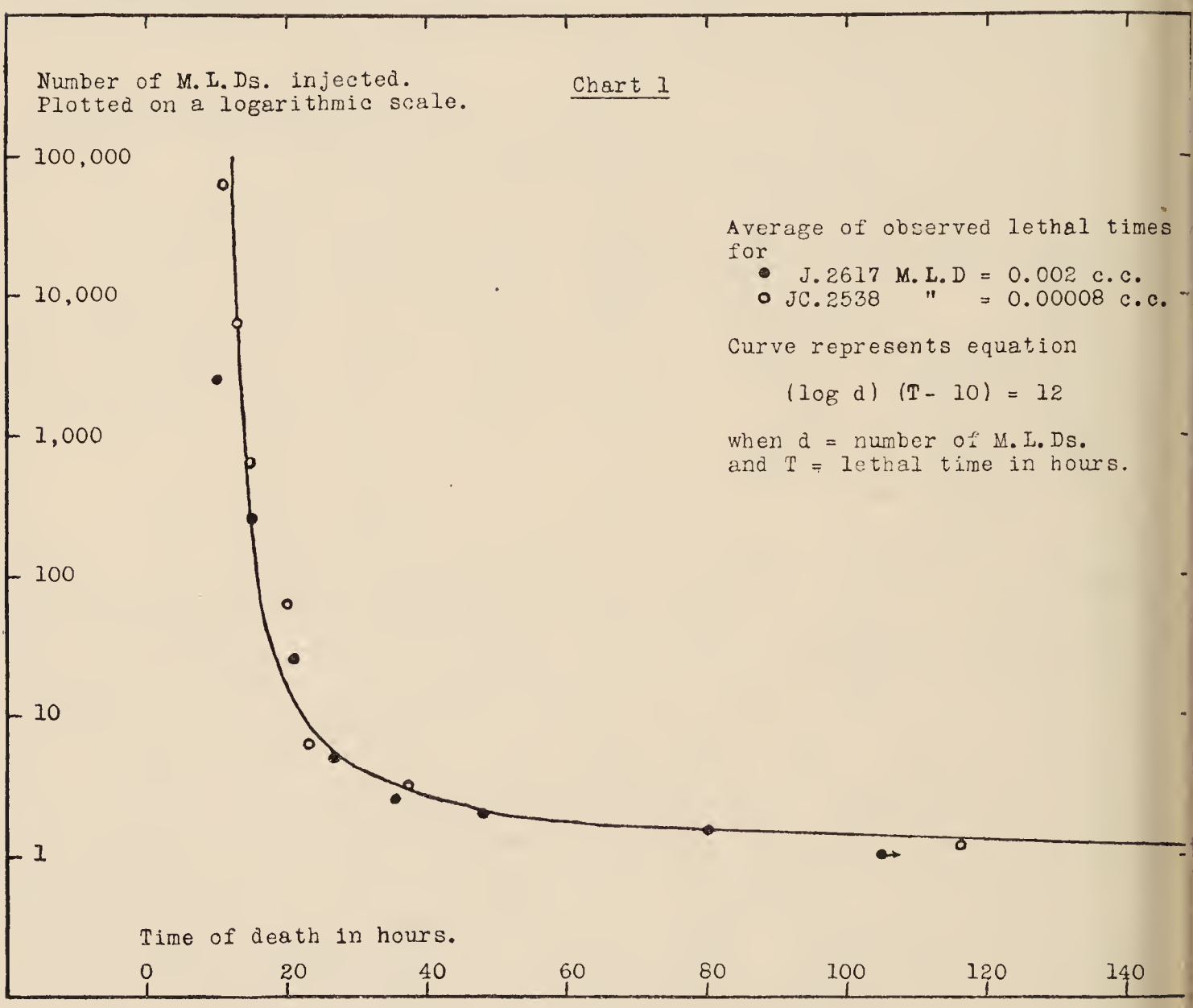
(1915). The M.L.D. of the concentrated toxin was taken to be 0·00008 c.c.: of 4 guinea-pigs injected with 0·000075 c.c. 1 died earlier than 5 days, 1 later and 2 survived: of 4 injected with 0·0001 c.c., 1 died at the end of 5 days, 2 earlier and 1 later. If chemically pure toxin could be prepared the constants of the equation connecting dosage with death-time would be the same however the toxin was prepared. But chemically pure toxin has never been prepared: the true toxin molecules probably constitute but a very small fraction of the purest toxin hitherto made. With moderately strong toxin non-specific effects, due to impurities present, may cause some discrepancy between observed and calculated lethal time but may not greatly affect the value of the constants. The formula for this concentrated toxin JC 2538 was therefore tried with the same constants as those used for the moderately strong toxin previously recorded: $(\log D - \log 0{\cdot}00008)(T-10)=12$ gave calculated times in close accord with observed times.

Chart 1 shows graphically how a single formula may connect dosage and death-time over a long range from 0 to over 60,000 fatal doses for two different toxins, one a routine batch of toxin with a minimal lethal dose of 0·002 c.c. and the other a concentrated toxin with an M.L.D. of 0·00008 c.c. The points marked on the chart represent the average of the observed lethal times; individual readings differed greatly from the average. The general formula reads:—

$$(\log d)(T-10)=12 \text{ or } T=10+\frac{12}{\log d}.$$

when T = lethal time in hours and d = number of theoretical minimal fatal doses, *i.e.* a figure slightly less than the M.L.D. because the latter is the dose killing in 5 days and the former in infinite time. According to the formula, M.L.D., *i.e.* the dose of toxin that will kill a guinea-pig in 5 days, is 1·285 times the theoretical minimal lethal dose; this is the value for d in the above formula when T = 120, (5 days).

A fresh toxin Y 47 was investigated; this toxin was extremely weak in specific toxicity and the minimal lethal dose was 0·2 c.c., consequently, non-specific substances may have interfered to a great



extent with the lethal effect of any given dose. Accepting 10 hours as the minimal lethal time for all batches of diphtheria toxin we find that observed lethal times show close agreement with those calculated according to the formula,

$$(\log D - \log 0{\cdot}2)\,(T - 10) = 22.$$

Sex does not appear to have any effect upon lethal time. Among the guinea-pigs recorded in tables I. II. and III., record was kept of the sex of 92; of these

21 bucks died earlier than average time.
25 „ later.
22 does „ earlier.
24 „ later.

TABLE III.

Observed lethal times in hours of 40 *guinea-pigs of* 250 *grams weight injected subcutaneously with different doses of diphtheria toxin* Y 47. *The average lethal times are compared with times calculated according to the formula* $\log\left(\frac{D}{0{\cdot}2}\right)(T-10)=22$.

Dose.	0·1.	0·15.	0·2.	0·3.	0·5.	1.	2	5.
Observed lethal times .	lived	lived	lived	lived	120·0	50·5	43·0	35·8
	,,	,,	,,	160·0	69·0	45·0	36·0	32·2
	,,	...	,,	75·0	66·0	39·2	28·4	31·6
	,,	...	96·0	...	65·0	38·0	28·2	29·3
	,,	...	...	...	55·2	...	...	25·3
	...	...	...	...	44·7	...	...	24·2
	...	...	...	...	43·7	...	...	23·7
	...	...	...	...	...	...	...	23·5
	...	...	...	...	...	...	...	23·2
	...	...	...	...	...	...	...	29·3
	...	...	...	...	...	...	...	19·5
Average lethal times . .	lived	lived	lived	117·5	66·3	43·2	33·9	27·0
Calculated lethal times .	,,	,,	,,	135·0	65·3	41·4	32·0	25·7

The effect of *dilution* was next studied. Pairs of male and female guinea-pigs of 250 grams weight were injected on the same day with doses of 0·0005 c.c., 0·05 c.c. and 0·5 c.c. of diphtheria toxin J 2617 contained in a total volume of 0·5 c.c. and of 5·0 c.c. From the results recorded in table IV. it is not possible to show that dilution has any effect.

TABLE IV.

Showing the lethal times of guinea-pigs of 250 *grams weight injected subcutaneously with various doses of diphtheria toxin* (J 2617) *in different concentrations.*

Dose.	Total volume injected in c.c.	Time of death of male guinea-pig (hours).	Time of death of female guinea-pig (hours).
0·005 c.c. .	diluted to 5·0	48·0	31·0
	,, 5·0	23·0	34·0
	,, 0·5	24·0	30·0
	,, 0·5	29·0	38·0
0·05 c.c. .	diluted to 5·0	23·0	23·5
	,, 5·0	20·7	13·0
	,, 0·5	21·0	12·0
	,, 0·5	22·2	21·0
0·5 c.c. .	diluted to 5·0	17·4	16·5
	,, 5·0	17·2	14·7
	undiluted 0·5	7·5	16·7
	,, 0·5	16·2	13·7

Dose and body weight.

In this paper an endeavour has been made to determine whether any formula can be applied that will connect dosage with weight. It is probable that the lethal times of older animals will not agree with

those of younger ones, even though the dose be adjusted for the weight. The general formula connecting dosage and death-time depends upon the general law governing the cause and effect and this law may be the same for all toxins and all animals, but the individual equation for a particular toxin and species of animal includes constants, the values of which are determined by the kind and strength of the toxin and the species of animals employed and must depend, among other factors, upon the rate of absorption and the resistance of the tissues to the particular toxin. These factors, and consequently the values of the constants may vary for the same toxin injected into animals of the same species but of different age and weight. If the values of the constants in the equation vary for animals of different ages, then it is impossible to adopt a simple formula giving correction for weight that would enable us to determine the dose which, when injected into an older animal of known weight, would kill that animal in the same time as a given dose would kill a smaller animal of standard weight.

It has usually been assumed that the lethal dose for any animal should be measured in terms of the two-thirds power of the weight or of the square root. Dreyer and Walker (1914) have claimed that the doses necessary to produce the same effect upon animals of different weight vary according to the surface areas of the animal, and that these areas are in proportion to $W^{0.72}$. In the following experiments use was made of their formula rather than the more vaguely determined formula giving dose in proportion to $W^{2/3}$. Table V. records the results of injecting 45 guinea-pigs weighing between

TABLE V.

Observed lethal times in hours, of 45 *guinea-pigs weighing from* 350-500 *grams injected with different doses of diphtheria toxin calculated according to the surface dose for the* 250 *gram guinea-pig.*

Corresponding dose.	0·001.	0·0015.	0·002.	0·003.	0·004.	0·005.	0·01.	0·05.	0·05.	5·0.
Observed lethal times	lived	lived	99·5	83·5	...	47·2	33·8	25·7	16·5	14·1
	,,	150·0	80·0	78·5	...	44·5	33·6	24·7	15·5	12·7
	,,	...	56·0	31·0	...	42·7	29·0	24·0	15·3	12·7
	150·0	...	48·2	28·3	...	41·0	26·0	21·5	...	10·6
	140·0	...	45·0	...	...	39·5	20·0	20·3	...	9·5
	...	...	...	...	...	27·3	...	...	...	9·4
	...	...	...	...	...	...	...	...	...	9·2
	...	...	...	...	...	...	...	...	...	9·2
	...	...	...	...	...	...	...	...	...	8·6
	...	...	...	...	...	...	...	...	...	7·5
Average lethal time for heavy guinea-pigs	–	–	65·7	55·3	...	40·4	28·5	23·2	15·8	10·3
Average lethal time for 250 gram guinea-pigs (see table I.)	–	–	105·4	80·1	...	35·7	27·2	21·1	15·3	10·8

350 and 500 grms. with doses of a moderately strong toxin calculated according to the formula $\left(\frac{W}{250}\right)^{0.72}$. The average lethal times for the guinea-pigs are compared with those of 250 gram guinea-pigs taken from table I. In the smaller doses the amount injected was too large; for example all 5 heavy guinea-pigs injected with a dose corresponding to one of 0·002 for a 250 gram guinea-pig died earlier than the average time for the 250 gram guinea-pig, whereas not one out of 8 guinea-pigs of 250 grams weight died earlier than the average of the 5 heavier guinea-pigs injected with the same surface doses. With multiple lethal doses there is close agreement between the lethal time for heavy and light guinea-pigs injected with the same surface dose, but this agreement is of little significance because large variations in multiple doses are needed to effect an appreciable difference in the lethal time.

A few heavy guinea-pigs were injected with surface doses of the weak toxin Y 47. The results recorded in table VI. show that there

TABLE VI.

Observed lethal times in hours, of a number of guinea-pigs (26) *weighing* 350-500 *grams injected with weak diphtheria toxin* (Y 47) *calculated according to the surface dose for a* 250 *gram guinea-pig.*

Corresponding dose.	0·1.	0·15.	0·2.	0·3.	0·5.	1·0.	2·0.	5·0.
Observed lethal times	lived	...	lived	88·7	87·0	48·0	46·8	38·5
	,,	...	,,	...	58·7	34·7	...	37·6
	,,	...	...	...	56·5	...	...	33·7
	...	...	...	...	50·5	...	...	32·3
	...	...	...	...	47·7	...	...	32·3
	...	...	...	...	...	...	...	31·2
	...	...	...	...	...	...	...	30·7
	...	...	...	...	...	...	...	29·7
	...	...	...	...	...	...	...	29·4
	...	...	...	...	...	...	...	29·0
	...	...	...	...	...	...	...	27·2
	...	...	...	...	...	...	...	27·0
Average lethal times for heavy guinea-pigs	...	...	–	(88·7)	60·1	41·3	(46·8)	31·5
Average lethal times for light guinea-pigs (see table III.)	...	...	–	135·0	65·3	41·4	32·0	25·7

may be close agreement in lethal times for guinea-pigs of different weights injected with certain surface doses but there appears a marked delay in time of death of the heavier guinea-pigs injected with a surface dose of 5 c.c. In this instance non-specific substances may have had a greater effect upon the smaller guinea-pigs.

The general conclusion is that for accurate work upon lethal dose it is essential to keep to a standard weight such as 250 grms., but for rough approximation larger animals may be used with an allowance for weight between $W^{\frac{1}{2}}$ and $W^{\frac{3}{4}}$.

Cobra venom.

An attempt was made to test whether the formula connecting dosage and death-time could be applied equally to cobra venom. I am indebted to Dr H. J. Sudmersen for injecting the animals recorded in the following experiments. Table VII. shows the time

TABLE VII.

Observed lethal times in minutes, of 17 *guinea-pigs of* 250 *grams weight injected with different doses of cobra venom. The average lethal times are compared with times calculated according to the formula* $(\log D - \log 0{\cdot}2)(T - 6) = 70$.

Dose.	0·6.	1·0.	2·0.	4·0.	10·0.
Observed lethal times	270·0	112·0	80·0	73·0	55·0
	...	100·0	80·0	62·0	54·0
	...	100·0	73·0	60·0	47·0
	...	...	70·0	50·0	47·0
	...	...	...	...	43·0
Average lethal times	(270·0)	104·0	75·7	61·2	49·2
Calculated lethal times	151·0	106·0	76·0	60·0	47·0

of death of 17 guinea-pigs of 250 grams weight injected with varying doses of cobra venom. The venom used had frequently been tested and the M.L.D was known to be approximately 0·2 mgrm. Close agreement is shown between the observed lethal times and those calculated according to the formula

$$(\log D - \log 0{\cdot}2)(T - 6) = 70.$$

when D is the dose in milligrams and T the lethal time in minutes. The *concentration* of venom has a great influence upon the death-time. Two guinea-pigs injected with 10 mgrms. in 5·0 c.c. died in 43 and 54 minutes while two others injected at the same time with 10 mgrms. in 0·5 c.c. died in 25 and 36 minutes. A further experiment with heavy guinea-pigs showed that injection in more concentrated solution reduced the lethal time from 56 and 59 minutes to 45 and 46 minutes. Three guinea-pigs injected with 100 mgrms. in 5 c.c. died in 10, 12 and 14 minutes: the calculated times for more diluted solutions is 26 minutes. It is probable that more concentrated solution of venom quickly breaks down capillary walls and the venom is more rapidly introduced into the blood stream. The early deaths of guinea-pigs injected with 100 mgrms. in concentrated solution justifies the choice of so short a minimal lethal time as 6 minutes.

Table VIII. shows the influence of *weight* or age. Even when dose has been calculated as directly proportional to weight, heavier guinea-pigs die later than those of 250 grms. Thus guinea-pigs weighing 500 grms. injected with 16·47 mgrms. of cobra venom (which is the surface dose equivalent to 10 mgrms. for guinea-pigs of 250 grms.)

did not die so quickly as the light guinea-pigs injected with 4 mgrms. No simple relationship between dosage of venom and weight is apparent in these experiments.

Table VIII.

Observed lethal times in minutes, of 21 *guinea-pigs weighing* 350-500 *grams injected with cobra venom, calculated according to the surface dose for a* 250 *gram guinea-pig.*

Dose.	0·6.	1·0.	2·0.	4·0.	10·0	100·0.
Observed lethal times .	1125·0	190·0	145·0	98·0	83·0	26·0
	...	154·0	140·0	90·0	78·0	22·0
	...	120·0	138·0	72·0	59·0	20·0
	...	...	100·0	...	57·0	20·0
	...	...	...	...	56·0	...
	...	...	...	...	33·0	...
Average lethal time for heavy guinea-pigs	(1125·0)	155·0	130·7	86·7	61·0	22·0
Average lethal time for light guinea-pigs (see table VII.)	(270·0)	104·0	75·7	61·2	49·2	14·0

Summary.

(1) The observed lethal times of guinea-pigs injected with a series of doses varying from 1 to 62,000 lethal doses of two different specimens of strong *diphtheria toxin* were found to be in close accord with lethal times calculated according to the formula $(\log d)(T - 10) = 12$ when d = number of theoretical minimal lethal doses, and T = lethal times in hours.

(2) The minimal lethal time for diphtheria toxin is 10 hours.

(3) For a very weak toxin it was found that while the delay in lethal time was still dependent upon the logarithm of the number of lethal doses, the value of the constant was increased, due probably to increased non-specific effects delaying the rates of absorption.

(4) For accurate work upon lethal dose it is essential to keep to some standard weight such as 250 grms., but for rough approximation larger animals may be used with an allowance for weight between $W^{\frac{1}{2}}$ and $W^{\frac{3}{4}}$. Sex did not appear to have any effect on lethal time.

(5) A sample of *cobra venom* was examined and the observed lethal times for a limited range of dosage (from 3 to 50 M.L.D.) agreed with lethal time calculated according to the formula

$$(\log d)(T - 6) = 70.$$

The theoretical minimal lethal time for cobra venom is taken as 6 minutes. Guinea-pigs injected with concentrated solutions die more rapidly than those injected with the same weight of venom in diluted solutions. No simple relationship between dosage of cobra venom and

weight of animal is apparent from the experiments. Guinea-pigs of 250 grams weight died earlier than guinea-pigs of twice the weight injected with four times the dose.

I am greatly indebted to Dr Major Greenwood for his kindness in reading through this paper and making many helpful criticisms and suggestions.

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